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EARLY PREDICTION FOR COMMON COMPLICATIONS OF LIVER CELL FAILURE USING FECAL CALPROTECTIN CONCENTRATION

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Original Article

ABSTRACT

Introduction: Liver cell failure complications especially spontaneous bacterial peritonitis (SBP) and hepatic encephalopathy (HE) are still major problem in early detection. Fecal calprotectin (FC) consider one of inflammatory markers of gastrointestinal (GIT) due to neutrophilic escape and migration so FC concentration is sensitive and non-invasive test to determine the active intestinal inflammatory related to SBP and HE.

Aim of the Work: The aim of this study is using fecal calprotectin (FC) for early prediction of onset and severity of SBP and HE.

Subjects and Methods: in this prospective controlled clinical trial sixty subjects and patients enrolled in our study 15 healthy non cirrhotic subjects, 15 cirrhotic patients without complications, 15 patients suffering from SBP and 15 with HE. Stool samples were collected for measuring FC by ELISA excluding patients with GIT inflammatory diseases and GIT bleeding.

Results: According to Child-Pugh and MELD scores for liver cell failure FC was elevated according to the severity of disease. FC in cirrhotic patients was (75.7± 36.8 mg/kg), SBP patients (195.3±88.3 mg/kg) and HE patients (349.7±106.4 mg/kg). Highly significant positive correlation founded between plasma venous ammonia and FC (P<0.001). Positive correlation emerging between elevated FC and HE grading as measured by West-Haven criteria as low grade HE is (258.7±41.9 mg/kg) and high grade HE is (429.4± 74.9 mg/kg). FC has sensitivity of 93.3% and specificity 66.7% in prediction of HE with positive predictive value (PPV) and negative predictive value (NPV) of 73.7% and 90.9% respectively at cut off value of > 205 mg/kg, on other hand FC has sensitivity of 73.3% and specificity 86.7% with PPV and NPV of 84.6% and 76.5% respectively at cut off value of >99 mg/kg in prediction of SBP patients.

Conclusion: Significant elevation in FC in cirrhotic patients with SBP and HE making it a simple promising non-invasive screening test for early prediction of these complications.

KEYWORDS: Fecal Calprotectin, Liver Cell Failure, Spontaneous Bacterial Peritonitis, Hepatic Encephalopathy

INTRODUCTION

Cirrhosis and chronic liver cell failure are leading causes of morbidity and mortality, with the majority of preventable cases attributed to viral hepatitis, excessive alcohol consumption, or nonalcoholic steatohepatitis. Cirrhosis often is an indolent disease; most patients remain asymptomatic until the occurrence of decompensation, characterized by ascites, spontaneous bacterial peritonitis, hepatic encephalopathy, or variceal bleeding from portal hypertension [1].

The gut flora and bacterial translocation causing intestinal inflammation which play important role in the

pathogenesis of hepatic encephalopathy (HE) and spontaneous bacterial peritonitis (SBP) [2].

SBP may be due to numerous alterations in intestinal flora, mucosal barrier functions and immunological defense mechanisms and altered portal circulation compromise the usual filtration function of the liver [3].

HE is a disturbance in central nervous system function occurs when the liver is no longer able to remove toxic substances in the blood. The gut flora and bacterial translocation also play a role in the pathogenesis of HE. Bacterial overgrowth is a responsible factor for minimal HE in cirrhotic patients [4].

Fecal Calprotectin is a calcium and zinc-binding protein, accounts for up to 60% of cytosolic protein in neutrophils [5].The presence of calprotectin in faeces quantitatively relates to neutrophils migration into the gastrointestinal (GI) tract and released into gastrointestinal lumen and serves as a useful biomarker for accurately identifying intestinal inflammation because it is released during cell activation and death [6]. Calprotectin is resistant to enzymatic degradation and can be easily measured in feces. Calprotectin might be a promising diagnostic parameter to diagnose the onset and course of HE and SBP due to presence of calprotectin in faeces.

So the aim of our study is to assess the role of fecal calprotectin (FC) as a useful biomarker for early diagnosis of SBP, early prediction of HE and its severity [7].

SUBJECTS AND METHODS

This prospective controlled clinical trial was conducted on 60 subjects who were admitted to Internal Medicine Department, Tanta University Hospitals after approval of ethical committee and written consent for all patients. They were divided into 15 healthy subjects as a control, 15 patients with liver cirrhosis without complications, 15 patients with liver cirrhosis complicated by SBP and 15 patients with liver cirrhosis complicated by HE.

The diagnosis of liver cirrhosis was based on clinical, laboratory and radiological findings. The disease severity was based on Child-Pugh Classification and MELD score [8]. Assessment of hepatic encephalopathy was calculated according to West-Haven criteria for hepatic encephalopathy [9]. The diagnosis of SBP in patients with ascites is established by definition as ascetic fluid neutrophils count of more than 250 cells/μL [2].

Patients with inflammatory bowel disease (IBD), coeliac disease, colorectal carcinoma, active GI bleeding, certain drugs [e.g. non-steroidal anti-inflammatory drugs (NSAIDs), anticoagulants, antibiotic therapy, proton pump inhibitors], and Alcohol abuse were excluded. All patients who reported about diarrhea were also excluded from this study.

After full history taking and complete clinical examination of all subjects, Routine laboratory investigations were done including white blood count (WBC),C-reactive protein (CRP), serum ammonia, liver function tests (LFT) including [serum bilirubin (total and direct), serum albumin, and prothrombin time] and kidney function tests including serum urea, serum creatinine and BUN. Ascetic fluid sample (30 ml) for all patients with liver cirrhosis and ascites was aspirated under complete aseptic conditions.

MEASUREMENT OF FECAL CALPROTECTIN CONCENTRATION

Fecal calprotectin was assayed by a commercial enzyme-linked immunosorbent assay (ELISA) method according to the manufacturer's instructions (Calprest, Dynex Elisa Eurospital, Trieste, Italy). Each subject provided a single stool sample collected within 48 h of admission to our hospital; samples obtained for FC measurement were collected in screw-caped disposable plastic containers and stored in aliquots at -20 0C until they were assayed. The FC concentration results

were expressed in mg of FC per kilogram of wet feces. Normal ranges according to the manufacturer's instructions: The median value in healthy adults is about 25 mg/kg. Samples giving values above 50 mg/kg are regarded as positive Calprest test.

STATISTICAL ANALYSIS

The collected data were tabulated and analyzed using SPSS version 17 software (SPSS Inc, Chicago, ILL Company). Quantitative data were expressed as mean and standard deviation. Comparison of continuous parametric data between more than two groups was made by using one way ANOVA with Tukey's post-test. Pearson test for correlations between different parametric parameters was used. ROC curve were used for estimation of sensitivity, specificity, cut off level, positive predictive value and negative predictive value. The accepted level of significance in this work was stated at 0.05 (P <0.05 was considered significant).

RESULTS

The study was enrolled 60 subjects who were divided into 15 healthy control subjects, 15 cirrhotic patients without complications, 15 cirrhotic patients complicated by SBP and 15 cirrhotic patients complicated by HE. Comparison between the studied groups as regard age and gender showed statistically insignificant difference.

The results of our study compared the studied groups as regard FC showed statistically highly significant difference (P<0.001) with lowest value in the control group (31.33 ± 8.62 mg/kg) and highest value in the HE group (349.73 ± 106.41 mg/kg) (Table 1) (Figure 1).

Comparison between cirrhotic, low grade HE and High grade HE groups as regard FC showed statistically highly significant difference (P<0.001) with lowest value in the cirrhotic group (75.67 ± 36.83 mg/kg) followed by low grade HE (258.71 ± 41.88 mg/kg) with highest value in the high grade HE group (429.38 ± 74.9 mg/kg) (Table 2). Our results showed significant difference between Child-Pugh score groups as regard fecal calprotectin levels (P<0.001) (Table 3).

Our results showed significant positive correlation between MELD score and fecal calprotectin (P<0.001) and significant positive correlation between serum ammonia and fecal calprotectin (P<0.001) (Figure 2 and 3).

ROC curve showed that FC had sensitivity of 93.3 % and specificity of 66.7 % in prediction of HE with PPV and NPV of 73.7% and 90.9% respectively at cut off value of > 205 mg/kg and as regard SBP, FC had sensitivity of 73.3% and specificity of 86.7% with PPV and NPV of 84.6% and 76.5 % respectively at cut off value 99 mg/kg (Table 4) (Figure 4 and 5).

Table 1: Comparison between Studied Groups as Regard Fecal Calprotectin

Groups	Fecal Calprotectin (Mg/Kg)		ANOVA
	Range	Mean ± SD	P-Value
Hepatic Encephalopathy (HE)	219 - 543	349.73 ± 106.41	<0.001*
Spontaneous bacterial peritonitis (SBP)	23 - 380	195.27 ± 88.27	
Cirrhosis	21 - 175	75.67 ± 36.83	
Control	18 - 48	31.33 ± 8.62	
Tukey's Test			
He &Sbp	He & Control	He & Cirrhosis	Sbp & Control	Sbp & Cirrhosis	Cirrhosis & Control
<0.001*	<0.001*	<0.001*	<0.001*	<0.001*	0.006*

*Significant <0.05 *highly significant <0.001

Table 2: Comparison between Cirrhotic, Low Grade HE and High Grade
HE Groups as Regard Fecal Calprotectin

	Fecal Calprotectin (Mg/Kg)		ANOVA
	Range	Mean ± SD	P-Value
Low grade Hepatic encephalopathy (HE)	219 - 345	258.71 ± 41.88	
High grade Hepatic encephalopathy (HE)	287 - 543	429.38 ± 74.90	<0.001*
Cirrhosis	21 - 175	75.67 ± 36.83	
Tukey's Test			
Low & High Grade HE		Low Grade HE & Cirrhosis	High Grade HE & Cirrhosis
<0.001*		<0.001*	<0.001*

*Significant <0.05 *highly significant <0.001

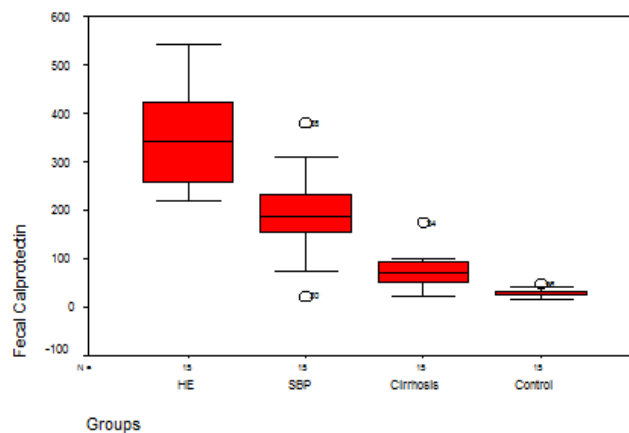**Table 3**: **Comparison between Fecal Calprotectin Levels in Different Child-Pugh Score**

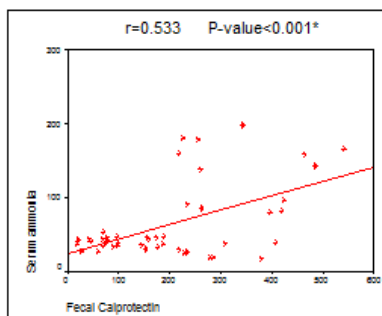| Child Score	Fecal Calprotectin		ANOVA
	Range	Mean ± SD	P-Value
A	21 - 219	96.28 ± 58.31	
B	75 - 543	212.33 ± 126.97	<0.001*
C	23 - 465	291 ± 134.88	
Tukey's Test			
A & B		A & C	B & C
<0.001*		<0.001*	<0.001*

*Significant <0.05 *highly significant <0.001

Table 4: Diagnostic Validity of Calprotectin in Prediction of HE And SBP

| ROC Curve between C And HE as Regard Calprotectin						
	Cut Off (Mg/Kg)	Sens.	Spec.	PPV	NPV	Accuracy
Prediction of HE	> 205 *	93.3%	66.7%	73.7%	90.9%	86%
Prediction of SBP	> 99 *	73.3%	86.7%	84.6%	76.5%	0.78%

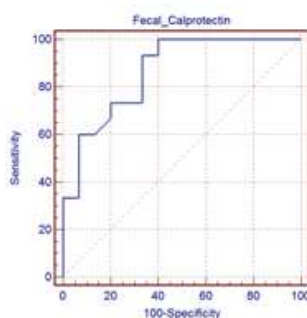**AUC:** Area under the curve **PPV:** Positive predictive value **NPV:** Negative predictive value

Figure 1: Comparison between Studied Groups as Regard Fecal Calprotectin by Box Plot
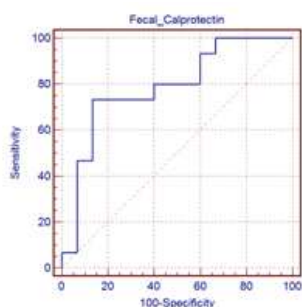
Figure 2: Correlation between Fecal Calprotectin and Serum Ammonia
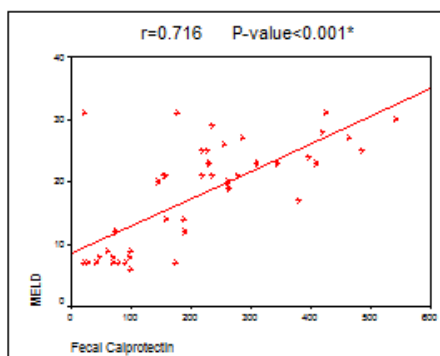
Figure 3: Correlation between Fecal Calprotectin and MELD Score

Figure 4: Roc Curve for Prediction of HE By Using Fecal Calprotectin

Figure 5: Roc Curve for Prediction of SBP by Using Fecal Calprotectin

DISCUSSIONS

Patients with liver cirrhosis have an increased risk of infections mainly SBP which is present in approximately 20% of patients with cirrhosis and ascites due to bacterial translocation and altered gut flora related to liver dysfunction

and reduced reticulo-endothelial function [2]. Small intestinal bacterial overgrowth is known to play an important role in the pathogenesis of cirrhosis complications such as HE, SBP and endotoxemia [10].

Calprotectin is a 36-kD constitutes approximately 60% of soluble cytosol proteins in human neutrophil granulocytes. Calprotectin is a surrogate marker of neutrophil turnover and is elevated in a number of inflammatory conditions [11]. As the GIT of cirrhotic patients shows various alterations of its mucosal barrier including infiltrates of neutrophils and the presence of fecal calprotectin quantitatively relates to intestinal neutrophil migration [12]. Therefore, fecal calprotectin might be a valid parameter to diagnose the onset and severity of HE and SBP in liver cirrhotic patients [13].

In present study, mean FC were significantly higher in cirrhotic patients compared with control group (P<0.001) despite of a careful exclusion of other causes of abnormal calprotectin results, e.g. GI bleeding, diarrhea, alcohol intake, and drugs like anticoagulants.

Likewise, Gundling et al. [14] who reported that fecal calprotectin levels were elevated in cirrhotic patients (65.8 mg/kg) compared to controls (17.5 mg/kg). It is also in agreement with Pleguezuelo M et al, [6] who found that calprotectin level is elevated in patients with liver cirrhosis but in faeces rather than in plasma.

In present study, patients were divided according to the severity of liver disease using modified Child Pugh score into three groups, child A, B and C. The elevated FC in this study showed a significantly positive correlation with the severity of liver disease (P<0.001). The corresponding correlation coefficients between MELD score and FC by Spearman's were 0.716 (P<0.001) respectively.

Fecal calprotectin in cirrhosis was firstly investigated by Yagmur et al. [15] who founded a significant elevation in FC in patients with advanced chronic liver disease (Child-C) and additionally added that there was a trend towards higher levels of fecal calprotectin in subjects with alcoholic cirrhosis. However, Montaltoet al. [16] who performed a longitudinal study of active-drinking alcoholics found no significant differences in median FC between alcoholics and controls. Homann et al. [17] stated also that plasma calprotectin concentrations were lower in viral liver disease than in non viral liver disease.

Homann et al. [17] investigated the prognostic value of plasma and ascites calprotectin in cirrhosis. They demonstrated that plasma calprotectin was a highly significant marker of poor survival in alcohol induced cirrhosis as high calprotectin concentrations were significantly associated with poor survival. The same authors found no association between increased plasma calprotectin concentrations and the severity of liver disease.

Yagmur et al. [15] stated that plasma level of calprotectin did not correlate with the severity of chronic liver diseases rather than alcoholic liver disease.

In present study we compared FC between control and cirrhotic subjects with and without SBP. Mean FC was higher when SBP was present and the difference was highly significant (P<0.001) with lowest value in the control group followed by cirrhotic with highest value in the SBP group and when we use FC in prediction of SBP, FC had sensitivity of 73.3% and specificity of 86.7% with PPV and NPV of 84.6% and 76.5 % respectively at cut off value 99 mg/kg.

Therefore, as Gundling et al. [14] concluded, FC may serve as a screening tool to identify cirrhotic patients with SBP. However, further studies are needed to investigate FC prospectively in cirrhotic patients with ascites and SBP before and after medical treatment in comparison to standard diagnostic procedures.

In present study, we compared FC between control, cirrhotic, SBP and HE groups showed high significant (P<0.001) with lowest value in the control group followed by cirrhotic followed by SBP with highest value in the HE group.

In our study, a significant association emerged between elevated FC and HE grading as measured by West-Haven criteria. The grade 0 and 1 (low grade HE) from 2, 3 and 4 (high grade HE) highly significant (P<0.001) with lowest value in the cirrhotic group (76±15 mg/kg) followed by low grade HE (239±19 mg/kg) with highest value in the high grade HE group (489±23mg/kg) and when we use FC in prediction of HE, FC had sensitivity of 93.3 % and specificity of 66.7 % in prediction of HE with PPV and NPV of 73.7% and 90.9% respectively at cut off value of > 205 mg/kg.

In our study, positive correlation of plasma venous ammonia and FCCs was significant (r= 0.533; P< 0.001). Therefore, FC may serve as a screening tool to identify cirrhotic patients with HE. Furthermore, assessment of FC may facilitate grading of HE severity which may be sometimes subjective when using clinical criteria only. Further studies are necessary to analyze fecal calprotectin prospectively under medical therapy in cirrhotic patients with HE in comparison to standard diagnostic procedures. Gundling et al. [14] found a significant between elevated FC and HE grading as measured by West-Haven criteria (P < 0.001).

Differentiating grading of 0 and 1 from 2 and 3 resulted in a sensitivity of 94% and a specificity of 95% using an optimal cut point 164 mg/kg (P < 0.001). And conclude that, FC may serve as a screening tool to identify cirrhotic patients with HE. The high level of FC in patients with HE may be explained by small intestine bacterial overgrowth (SIBO), Gupta et al. [18] studied the role of bacterial overgrowth of the small intestine among patients with minimal HE. 55.9% of patients with cirrhosis had minimal HE, among these patients 38.6% had SIBO, while 8.9% of patients without Minimal HE had SIBO (P<0.001). The prevalence of SIBO was higher in patients with Child- Pugh classes B and C (69.2%) compared to those in class A (30.8%).

CONCLUSIONS

Fecal calprotectin is significantly elevated in cirrhotic patients dependent on the severity of liver disease and have a significant correlation emerged between its elevated and hepatic encephalopathy and spontaneous bacterial peritonitis so Fecal calprotectin is a valid parameter as it is a simple, non-invasive and rapid screening test to make a diagnosis and assessment of the severity of these complications.

CONFLICT OF INTEREST

We (the authors) declare that we have no conflict of interest.

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